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SERINE ELASTASE AND MATRIX METALLOPROTEINASE (MMP) INHIBITION INDUCES PULMONARY ARTERY (PA) SMOOTH MUSCLE CELL (SMC) APOPTOSIS LEADING TO REGRESSION OF VASCULAR HYPERTROPHY ((K.N. Cowan, P.L. Jones, and M. Rabinovitch)) Department of Pathology, University of Toronto and the Division of Cardiovascular Research, Hospital for Sick Children, Toronto, ON

Increases in elastolytic activity and tenascin-C (TN) deposition are associated with the progression of pulmonary vascular disease. Previous studies have shown that TN accumulation is positively regulated by MMP activity which is elevated on attached collagen and supports rat PA SMC survival and growth factor-dependent proliferation. Conversely, on floating collagen suppressed MMP activity decreases TN and leads to apoptosis. To determine whether rat PA SMCs in their native environment respond similarly, and whether regression of vascular lesions may occur through inhibition of MMPs and elastases, implicated in MMP activation, we cultured hypertrophied rat PAs on attached collagen gels supplemented with media containing protease inhibitors, or on floating gels. Control PAs on attached collagen displayed progressive medial hypertrophy (1.2 fold) and high levels of elastolytic activity (1.4 fold*, in comparison to floated PAs; *=$p<0.05$) by elastase assay assessed by the degradation of [^{3}H]-elastin. MMP-2 activity was also increased (1.7 fold compared to floating PAs by gelatin zymography and western immunoblotting). This proteolytic environment was associated with increased TN (1.4 fold) determined by immunostaining and northern blotting and induction of vascular cell proliferation (3.8 fold*) by immunostaining for the proliferating cell nuclear antigen. Conversely, regression of hypertrophy (2 fold*) occurred through MMP or elastase inhibition either endogenously suppressed on floating collagen, or by using either the MMP inhibitor GM-6001 (Glycomed), the serine proteinase inhibitor α1-proteinase inhibitor (α1-PI), or compound 1K (patent # Zeneca WO96/23512, 1996) a neutrophil elastase inhibitor (Veale et al, J Med Chem, submitted). Protease inhibition was associated with suppression of TN (4.9 fold*), decreased proliferation (20.8 fold*), and induction of apoptosis (7.8 fold* by TUNEL assay). We suggest that withdrawal of TN occurs via inhibition of proteinases and initiates apoptosis.